B_{reg}-ging rights

By Lev Osherovich, Senior Writer

Duke University School of Medicine researchers have developed a way to grow large quantities of regulatory B cells.¹ The team used an infusion of B_{reg} cells to suppress a murine form of multiple sclerosis and now wants to test the immunomodulatory cells in other autoimmune diseases. They are hoping to launch a company based on the technology.

B_{reg} cells produce the anti-inflammatory cytokine IL-10 and can counteract excessive immune activation in autoimmune and inflammatory diseases.²

In previous studies, a team led by Thomas Tedder, professor of research in immunology at Duke, engineered mice deficient in B_{reg} cells and showed that the animals were more susceptible to autoimmune and inflammatory diseases than wild-type animals.³ However, because B_{reg} cells constitute only a tiny fraction of circulating B cells, it was not possible to harvest enough cells to test whether infusing B_{reg}-deficient animals with B_{reg} cells would have a beneficial effect.

The new work by Tedder's team solves the B_{reg} cell supply problem. The researchers determined the conditions that promote the development of B_{reg} cells in the spleens of mice and reproduced those conditions *ex vivo* to grow large numbers of the cells.

"We can now identify subsets of B_{reg} cell precursors, purify that subset and adoptively transfer it" to mice unable to make their own B_{reg} cells, said Tedder. "If you want to treat autoimmune disease, being able to expand these cells is an advance."

Isolate and activate

The team began by purifying general B cell precursors from the spleens of mice and treating them *in vitro* with a range of cytokines suspected to influence B_{reg} cell development.

B cell precursors activated with IL-21 secreted IL-10, whereas controls treated with media or with other cytokines did not. When transferred into B_{reg}-deficient mice, the *in vitro*-activated cells decreased symptoms of experimental autoimmune encephalitis (EAE) compared with unactivated B cell precursors.

Because B cell activation typically is enhanced by T helper cells, the natural source of IL-21, the team suspected that other T cell molecules might further promote B_{reg} cell activation and growth. Indeed, IL-21 activation of B_{reg} cells was further increased by CD40, a B cell co-receptor that binds to CD40 ligand (CD40LG; CD40L; CD154), found on the surface of T cells.

Using this information, the researchers developed a streamlined procedure for growing B_{reg} cells *in vitro*. They transfected a fibroblast cell line

with CD154 and BlyS (BAFF), a general B cell-activating protein, and then cocultured B cell precursors with these fibroblasts in the presence of IL-21.

The resulting number of activated B_{reg} cells was about four million times the initial number of inactive cells. Reinfusion of those cells prevented the initial onset of EAE and decreased disease severity in mice that already had developed the condition compared with no treatment.

Results were published in *Nature*.

"The novel finding here is the ability to expand murine regulatory B cells in cell culture," said Abdolmohamad Rostami, professor of neurology at Thomas Jefferson University. "The next step is to extend this observation into humans—they should take whole B cells and treat them with human IL-21."

B_{reg}-adoon

Tedder thinks infusions of large numbers of patient-derived, *ex vivo*-activated B_{reg} cells could help to rapidly shut down autoimmune diseases.

He said B_{reg} cells specific for autoimmunity-associated antigens already are present in patients with autoimmune disease but that there are too few of them to affect disease. The hope is that boosting overall levels of B_{reg} cells would tip the balance of immune activity toward suppression of autoimmunity.

"Nature has already created a set of B_{reg} cells with immunomodulatory activity," said Tedder. "If you have an autoimmune disease, these cells are already responding. Our job is just to expand them."

Tedder noted that because B_{reg} cells act in an antigen-specific manner, there is little danger of the broad immunosuppression that is a concern with immunomodulatory therapies.

The team "did show that the mice were not immunosuppressed, which is a major problem with many medications for autoimmune disease," said Rostami. "With conventional immunosuppression, there is a risk that you would suppress the suppressor cells, too."

The challenge, Tedder said, is to grow enough of the human cells *in vitro* to achieve the kind of disease-suppressing effects seen in mice. Tedder said his laboratory is now working on reproducing and scaling up the B_{reg} cell expansion procedure with human cells, which grow more slowly than mouse cells.

Both Tedder and Rostami said the technique will allow researchers to compare B_{reg} cells head-to-head with T_{reg} cells, another type of immunomodulatory cell.

Last year, a trio of academic teams reported procedures for amplifying and reinfusing human T_{reg} cells in humanized mouse models of autoimmune disease.⁴⁻⁷

Tedder thinks T_{reg} cells might be best suited for chronic inflammatory conditions, whereas B_{reg} cells appear to be most effective in acute conditions.

His technique is patented and is available for licensing. Tedder said he is hoping to launch a company based on the technology and is in the process of finding investors and commercial development partners.

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COMPANIES AND INSTITUTIONS MENTIONED

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